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Environment-Spatial Conditional Learning in Rats With Selective Lesions of Medial Septal Cholinergic Neurons

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ABSTRACT: Cholinergic medial septal neurons may regulate several aspects of hippocampal function, including place field stability and spatial working memory. Monkeys with damage to septal cholinergic neurons are impaired in visual-spatial conditional learning tasks; however, this candidate function of septal cholinergic neurons has not been studied extensively in the rat. In the present study, rats with selective lesions of cholinergic neurons in the medial septum and vertical limb of the diagonal band of Broca (MS/VDB), made with 192 IgG-saporin, were tested on a conditional associative learning task. In this task, which we term "environment-spatial" conditional learning, the correct location of a spatial response depended on the array of local environmental cues. MS/VDB-lesioned rats were impaired when the two parts of the conditional problem were presented concurrently, but not when one environment had been learned before the full conditional problem was presented. Our findings suggest that cholinergic MS/VDB neurons participate in some aspects of conditional associative learning in rats. They may also shed light on the involvement of cholinergic projections to the hippocampus in modulating and remodeling hippocampal spatial representations.

KEY WORDS: medial septum; spatial learning; basal forebrain; acetylcholine; conditional associative learning; 192 IgG-saporin

INTRODUCTION

The medial septum and vertical limb of the diagonal band of Broca (MS/VDB) of the rodent and primate brain contain a large number of cholinergic neurons that project to the hippocampus (Lewis et al., 1967; Amaral and Kurz, 1985; Rye et al., 1984). Disruption of the MS/VDB by neurotoxic lesions or inactivation produces deficits in spatial working memory (Olton et al., 1978; Rashidy-Pour et al., 1996; Mizumori et al., 1990; Walsh et al., 1998) and in hippocampal theta rhythms (Bland, 1986; Vanderwolf, 1969). In contrast, studies with the selective immunotoxin, 192-IgG saporin (Wiley et al., 1991) have provided less conclusive evidence about a specific role for MS/VDB cholinergic neurons in spatial working memory function. Some studies have reported impaired spatial working memory after lesions limited to MS/VDB cholinergic neurons (Walsh et al., 1996; Shen et al., 1996; Leanza et al., 1996; Janis et al., 1998), whereas others have reported no impairment after selective loss of MS/VDB cholinergic neurons (McMahan et al., 1997; Chappell et al., 1998; Pang and Nocera, 1999). In turn, it has been suggested that more extensive damage to the basal forebrain, beyond the MS/VDB, is required to produce impairment in spatial working memory (Wrenn et al., 1999).

This discrepancy is somewhat surprising, given that electrophysiological recording studies provide strong evidence for a role of cholinergic MS/VDB neurons in spatial memory. For example, Shapiro et al. (1989) recorded place cell activity while rats with fimbria-fornix lesions explored an 8-arm radial maze. Hippocampal cells in these rats, when compared to controls, showed less spatial specificity, exhibiting place fields that were larger in size and less consistent across exposures to the maze. In addition, these rats performed poorly on rotated maze trials in which they had to use the same extra-maze visual cues to guide them. The "place fields" of hippocampal neurons in fimbria-fornix lesioned rats, in contrast, seemed to be more rigidly fixed to intra-maze cues. As evidence that these deficits were cholinergic, these investigators grafted undifferentiated basal forebrain neurons from fetal rats into the hippocampus of fornix-lesioned rats. The degree to which the grafts innervated, or connected the medial septum to the hippocampus, was directly correlated with the amount of improvement (toward normal place field structure and behavior) exhibited by each rat. In another study, MS/VDB quinolinic acid lesions produced a disruption of hippocampal place cell activity in rats during 8-arm radial maze exploration similar to that caused by fimbria-fornix lesions (Leutgeb and Mizumori, 1999). Furthermore, when control and MS/VDB lesion rats were subsequently put into a novel environment, consisting of the same 8-arm radial maze but with novel visual cues, the place fields of control rats readily remapped, whereas the septal lesioned rats had few redistributed place cells. Thus, it appears that the result of MS/VDB lesions is an excessively stable place field map. Again, this difference in neuronal activity corresponded to a difference in behavioral performance (Leutgeb and Mizumori, 1999). A similar effect on hippocampal place cell activity was also reported in rats with specific cholinergic medial septal lesions using 192-IgG saporin (Ikonen et al., 2002). Although selective cholinergic lesions were not associated with disruptions in place cell properties (e.g., spatial specificity) per se, this study again indicated that lesioned rats were more likely to maintain an established representation of a location despite changing environments. In particular, the septal lesioned rats were less likely to reorganize place fields despite going from a cy-

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lindrical to a hexagon-shaped container, two visually and geometrically distinct environments. Taken together, these data suggest that cholinergic input from the MS/VDB to the hippocampus is not required for formation of place fields, but is required for aspects of their modification and remapping.

Although place fields are a representation of specific place, they do not necessarily reflect the physical space the rat occupies per se, and should be thought of as memory maps rather than isomorphic maps of physical space (Shapiro and Eichenbaum, 1999; Eichenbaum et al., 1999). Hence, the role of cholinergic medial septal neurons may be in a specific type of spatial memory that requires rats to use place cell plasticity to "switch" from one environment to another in order to properly execute a reward-obtaining strategy. The persistent stability of the medial septal-controlled place fields in the hippocampus of 192 IgG-saporin lesioned rats may explain why lesioned rats are not impaired at learning a spatial working memory rule in only one environment. However, this observation suggests that impairments would be observed when a requirement to rapidly switch between place representations is instituted.

Indeed, evidence that the medial septal area is involved in spatial memory flexibility has come from nonhuman primate studies and appears to complement rat studies. Marmoset monkeys with excitotoxic lesions of the septum/diagonal band or hippocampal area CA1, or fimbria-fornix section (Ridley et al., 1989, 1991, 1992, 1996), were significantly impaired on a conditional visual-spatial discrimination task. The visual-spatial conditional discrimination task consisted of two identical object pairs (AA and BB) that were presented in pseudo-random alternations. When pair AA was presented, a choice of the left object of the pair was correct; when pair BB was presented, the choice of the right object of the pair was correct. The effect of lesion could be abolished by administering a cholinergic receptor agonist, pilocarpine (Ridley et al., 1989, 1991), or by fetal cholinergic tissue transplants into either the fimbria-fornix area itself (Ridley et al., 1992) or the hippocampus (Ridley et al., 1991). Additionally, specific cholinergic immunotoxin (ME20.4-IgG-saporin) lesions of the diagonal band also impaired conditional visual-spatial discriminations (Ridley et al., 1999). However, when the concurrent discrimination task was prefaced with a period in which the monkeys received alternating blocks of either stimulus pair, eliminating a requirement for rapid switching, no medial septal lesion impairment was observed (Ridley and Baker, 1997). We hasten to add, however, that impairment on visual-spatial conditional discriminations does not always follow damage to the fornix, even when hippocampal lesions produce impairment on the same tasks (Sziklas et al., 1996, 1998; Sziklas and Petrides, 2002). This may indicate that there are some conditions under which input to the hippocampus from the fornix is not required for conditional learning.

Based on the foregoing studies, we hypothesized that a task that required rats to use two spatial representations concurrently, requiring efficient "switching" of place field maps, would be sensitive to loss of MS/VDB cholinergic neurons. To this end, we selectively destroyed the cholinergic neurons in the MS/VDB in rats with microinjections of 192 IgG-saporin (Wiley et al., 1991; Heckers et al., 1994) and examined postoperative performance on a conditional visual-spatial associative learning task. The task, which we term "environment-spatial conditional learning," used a similar behavioral setup to that described by Ikonen et al. (2002). Two environments, an octagon and a cube, were constructed with different visual cues on the walls of each. The location of reward differed between the two environments. Control and MS/VDB-lesioned rats were trained on one of the environments and then introduced to the second environment for discrimination (Experiment 1), or were trained on both environments concurrently (Experiment 2).

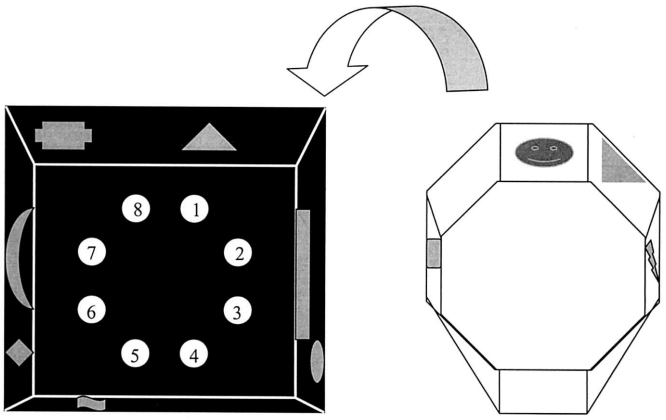
MATERIALS AND METHODS

Subjects

Forty-nine male Long-Evans rats from Taconic breeding colony (Germantown, NY) weighed 300–350 g at the beginning of the experiment and were housed at 22°C on a 12/12-h light/dark cycle (lights on at 0800 h). The rats had free access to water and food until 2 weeks postsurgery. At this point, they were placed on a restricted feeding schedule to reduce them to 85% of their free-feeding baseline weight. Each rat was individually housed and handled daily for 10 days before behavioral testing, thus familiarizing them with their primary handler. Their treatment complied with federal, state, and local guidelines. The animal facilities at Harvard University are fully AAALAC-accredited, and all animal protocols were approved by the Harvard University Standing Committee on the Use of Animals in Research and Teaching.

Surgery

Rats were divided into two groups: those receiving cholinergic medial septal lesions (MS/VDB, n = 24) and surgical controls (CONT, n = 25). Prior to surgery, each rat was deeply anesthetized by an intramuscular (i.m.) injection of a ketamine/xylazine mixture (80 mg/kg ketamine and 5 mg/kg xylazine; Phoenix Pharmaceuticals, St. Joseph, MO). They were also given atropine (0.25–0.3 ml of a 54 mg/ml solution i.p.; Phoenix Pharmaceuticals), to reduce salivation. Additional ketamine (10 mg, i.m.) was given every 45 min to maintain anesthesia. The rat's head was then shaved and placed in a stereotactic headholder (Kopf Instruments, Tujunga, CA) with the nose bar 3.3 mm below the interaural line. The skin overlaying the skull was disinfected with povidone-iodine solution (Betadine). The scalp was cut minimally with a single incision to expose the skull. Drill holes large enough to accommodate a 28-gauge needle were made at +0.45 mm anterior and ±0.6 mm lateral to bregma (coordinates taken from Baxter et al., 1995). In each of these two sites, injections of 192-IgG saporin (0.12 μg/μl; Chemicon, Temecula, CA) for lesion surgeries, or sterile phosphate-buffered saline for control surgeries, were placed at two positions: 0.3 μl at −7.8mm and 0.2 μl at −6.2 mm relative to the skull surface measured at bregma (flow rate 0.05 μl/min, for both). The needle was left in place for an additional 9 min and 6 min, respectively, after completion of the injection, to allow the toxin to diffuse from the injection site. Afterward, the scalp was sutured with Vicryl 3-0 sutures (Ethicon), and an antibiotic ointment with pain reliever was applied to the wound. Rats were given intraperi-



A. B.

FIGURE 1. Behavioral testing apparatus for Experiments 1 and 2. A: The eight circles in the center of the black floor designate the cups filled with Froot Loop-scented bedding. The rat would always begin a trial in the center of the eight cups and the orientation in which he was put in varied in a pseudo-random order. In this environment, cup 2 always contained the food reward. B: Eight-panel white foamboard piece that was inserted into the free-standing Plexiglas cube to make the "octagon" environment. In this environment, cup 4 always contained the food reward. The eight-panel piece was always placed in the cube with the same orientation.

toneal injections of electrolyte fluids (1 ml) for rehydration and intramuscular injections of Banamine (flunixin meglumine, 0.5 mg in 0.1 ml, Schering-Plough Animal Health, Union, NJ) as an analgesic. Additionally, Banamine (0.5 mg, i.m. daily) was given for 2 consecutive days postoperatively, to aid in recovery, and food was saturated with water to help the animals rehydrate. A minimum of 2 weeks was given for full recovery.

Apparatus

A black Plexiglas cube (1.5 m × 1.5 m) with an open top was decorated with distinctive visual stimuli on all four of its walls (e.g., one face contained a pattern drawn on cardboard and a cloth). Eight clear plastic cups, 6.5 cm in diameter and 8 cm in height, filled with Froot Loop-scented bedding, were placed in a symmetrical circle 45 cm in diameter in the center of the Plexiglas cube and arbitrarily numbered 1–8 consecutively. Froot Loop-scented bedding, made by crushing Froot Loops with corncob bedding, was used so that the rats could not identify the reward cup based on odor cues. The buried reward, a Froot Loop, was always located in the cup 2 in the cube environment. A white foamboard octagon, fitted precisely to the dimensions of the Plexiglas cube, served as a second set of local environmental cues. The cardboard was also decorated with unique visual stimuli, different from those in the cube. When inserted into the Plexiglas cube, it created an octagon that surrounded the same 8 cups in the center; cup 4 was arbitrarily chosen to be the reward center in this environment. The octagon was always inserted into the Plexiglas cube in the same orientation relative to the cube and to the external environment (Fig. 1).

Pretraining

All rats (CONT n = 25; MS/VDB n = 24) were not previously used on any behavioral tasks, and were introduced to Froot Loops in their home cage at least a week prior to testing. Four days prior to testing, cups filled with Froot Loop-scented rat bedding and each containing a buried Froot Loop, like those found in the testing apparatus, were put into each rat's cage in order to teach them to dig for a reward. Once each rat dug immediately after the cup was put into the cage, it was habituated to one of the two environments. Habituation involved five 10-min sessions during which the rat was allowed to explore the environment freely. No cups were present during this time. The floor of the environment was wiped down with 50% ethanol after each session under all conditions. The environment in which the rats were habituated would go on to be the one in which they were first tested. Habituation and subsequent initial testing in either the octagon or the cube was counterbalanced across lesion conditions.

Experiment 1: Behavioral testing procedure

One group of rats (CONT n = 10; MS/VDB n = 10) was first trained for 7 days (5 trials/day) to find the hidden Froot Loop in the designated cup in one of the environments (the simple spatial learning phase-part 1). Each rat was placed facing a different direction (north, south, east or west) in the center of the circle of cups at the beginning of each trial. Under all experimental conditions using cups, cups were rotated randomly after each trial and cleaned with 50% ethanol after each day of testing. Furthermore, fresh Froot Loop scented bedding was added to each cup at the beginning of each day to prevent a olfactory cues associated with baited cups from developing. After 7 days, at which time rats in both groups were making an average of less than 1 error per trial, the novel environment was introduced. Rats were trained for a further 8 days (10 trials/day) with both environments presented concurrently, 5 trials of each environment on each day, pseudo-randomly varied across trials (the conditional discrimination). Digging was defined as the rat using both front paws to sift through the scented bedding. Errors were recorded as cups in which the rats dug in with both paws, but did not contain a Froot Loop. Revisits to incorrect cup were not scored as errors. Because the rats were allowed to visit cups until they located the correct one, we viewed revisits to incorrect cups as analogous to correction trials; furthermore, this procedure was intended to limit any apparent lesion effects to discrimination performance. Repeat visits to cups were rare except on the first day of behavioral testing; this scoring procedure also avoided distortion of learning curves from the first day of training, while the rats were still becoming familiar with testing.

Experiment 2: Behavioral testing procedure

A second group of rats (CONT n = 15; MS/VDB n = 14) began testing in the conditional discrimination phase, without learning the discrimination in a single environment first. This design was identical to Experiment 1, except that the 7 days of training in a single environment were omitted. Rats were trained for 10 trials/day for 8 days with the square and octagon environ-

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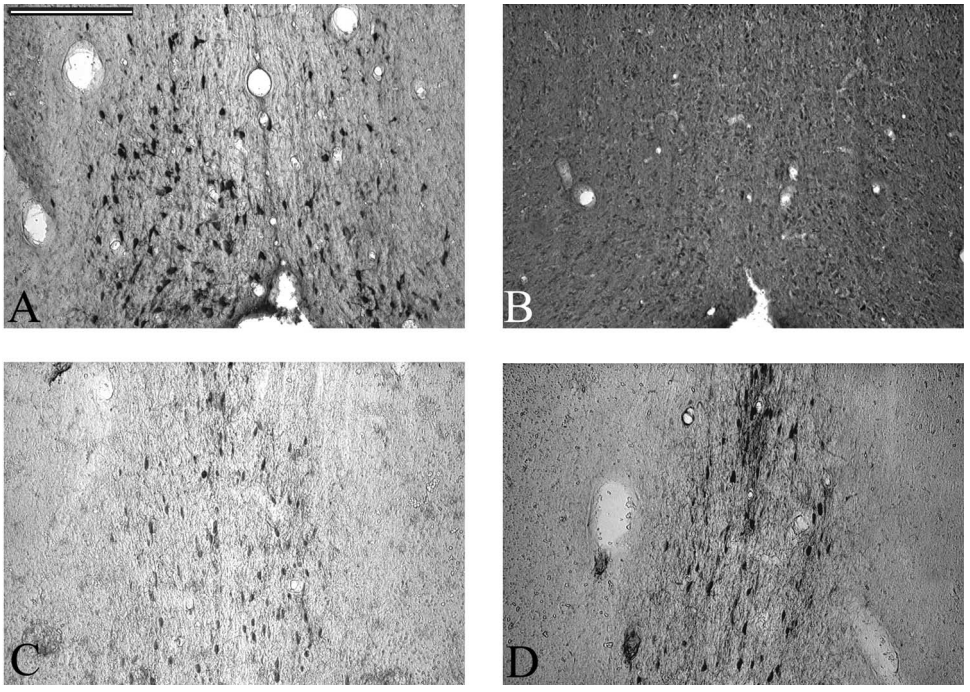

FIGURE 2. Immunohistochemistry for choline acetyltransferase (ChAT; A,B) and parvalbumin (C,D) in control (A,C) and medial septum and vertical limb of the diagonal band of Broca (MS/VDB)-lesioned (B,D) rats. The MS/VDB is devoid of cholinergic neurons in 192 IgG-saporin lesioned rats (B), compared to control rats (A). Parvalbumin immunostaining is intact in the MS/VDB of lesioned rats (D), supporting the specificity of 192 IgG-saporin lesions for cholinergic neurons. The scale bar in A (20 μm) applies to all panels.

ments pseudo-randomly varied across trials. Again, each rat was placed facing a different direction (north, south, east, or west) in the center of the circle of cups during the initiation of each trial, and error recording was identical to that in Experiment 1. In both experiments, the dependent variable was the total number of errors committed during each session of testing. Repeat visits to a cup during a trial were not counted as additional errors.

Histological Analysis

Some of the rats in these experiments participated in another behavioral study after the experiments reported here, but prior to sacrifice; rats were euthanized between 3 and 9 months postsurgery. Rats were given a lethal dose of Nembutal (100 mg/kg, Abbott Laboratories, North Chicago, IL). After induction of terminal anesthesia, the rat was transcardially perfused with 0.9% saline for 5 min, followed by 4% freshly depolymerized paraformaldehyde in 0.1 M phosphate buffer for 20 min, both at a flow rate of 18 ml/min. The brain was then extracted and immersed in 4% paraformaldehyde for another 2 h. Afterward it was immersed in 20% sucrose solution for 3 days.

After 3 days in sucrose, 60-μm coronal sections through the MS/VDB were taken on a freezing-sliding microtome and stored in 0.1 M phosphate-buffered saline (PBS) until staining. All cases were processed for choline acetyltransferase (ChAT) immunohistochemistry to detect the presence or absence of cholinergic neurons in the MS/VDB. A limited number of cases (MS/VDB n = 2, CONT n = 2) were also processed for parvalbumin immunohistochemistry, to confirm the selectivity of the immunotoxic lesion. (Because we routinely find that immunotoxic lesions in our laboratory do not damage parvalbumin-immunoreactive MS/VDB neurons, we did not consider it necessary to perform the second stain on all cases. Furthermore, any nonspecific damage or tissue necrosis that might be observed could be detected in the ChAT-immunostained sections.) Immunohistochemical procedures used goat polyclonal anti-ChAT (Chemicon AB144P, Temecula, CA) or mouse monoclonal anti-parvalbumin (Sigma Chemical, St. Louis, MO) primary antibodies and followed standard avidin-biotin complex staining procedures (e.g., Berger-Sweeney et al., 2000; Cahill and Baxter, 2001).

RESULTS

Immunohistochemical Analysis

All MS/VDB-lesioned animals included in this study had complete medial septal cholinergic lesions as verified by ChAT immunostaining (cf. staining in control rats, Fig. 2A, to staining in MS/VDB-lesioned rats, Fig. 2B). Lesions for all rats included in the two experiments were of similar extent, comprising cholinergic neurons throughout the rostrocaudal extent of the MS/VDB. Although we did not perform any quantitative neurochemical analyses on rats from these experiments, the lesions were similar in extent to those in other studies from our laboratory in which quantitative analyses were performed, which confirmed a massive depletion of cholinergic enzyme activity in hippocampus (>80%; e.g., Cahill and Baxter, 2001). Neither control nor lesion rats had

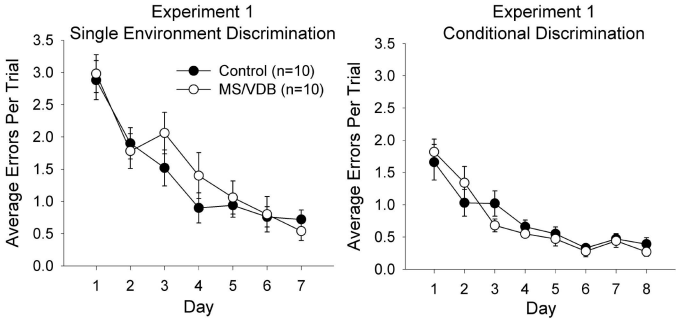

FIGURE 3. Results of Experiment 1. A: Single-environment learning. Medial septum and vertical limb of the diagonal band of Broca (MS/VDB)-lesioned and control rats learn the location of reward in a single environment at similar rates. B: Conditional discrimination. When a second environment is introduced, MS/VDB-lesioned and control rats learn the environment-spatial discrimination at similar rates.

damage to adjacent brain areas containing cholinergic neurons, such as the caudate-putamen. Staining for parvalbumin-immunoreactive (GABAergic; Freund, 1989) neurons in the MS/VDB was indistiguishable between control (Fig. 2C) and MS/VDB-lesioned (Fig. 2D) rats. This verifies the cholinergic specificity of 192 IgG-saporin.

Experiment 1: Single environment learning

There was no significant difference between control and MS/VDB-lesioned rats in learning the location of the reward in the single environment (Fig. 3A). A two-way analysis of variance (ANOVA) with lesion as a between-subject factor and session as a within-subject factor revealed a significant effect of session ($F(6,108) = 30.32$, $P < 0.0005$) but no effect of lesion ($F(1,18) = 0.35$, $P = 0.56$) or session by lesion interaction ($F(6, 108) = 0.92$, $P = 0.48$). To confirm the absence of a lesion effect we also compared the average number of errors per session for control and MS/VDB-lesioned rats, which also revealed no differences between the groups ($|t|s(18) < 1.27$, $P > 0.22$). We also compared performance between the two environments, which revealed no overall effect of environment ($F(1, 18) = 0.072$, $P = 0.79$) or environment by session interaction ($F(6, 108) = 0.948$, $P = 0.434$). Neither of these effects interacted with lesion (session by lesion $F(6,96) = 1.02$, $P = 0.41$ and environment by lesion $F(1,16) = 0.001$, $P = 0.98$). Hence, there were no differences based on whether the cube or octagon was the initial training environment.

Experiment 1: Conditional associative learning

When a novel environment was introduced concurrently with the previously learned environment to provide a conditional discrimination, no lesion effect on conditional associative learning was seen (Fig. 3B). A two-way ANOVA, with session as a within-subject factor and lesion as a between-subject factor, showed no significant effect of lesion ($F(1, 18) = 0.06$, $P = 0.802$). Again, an overall effect of session ($F(7, 126) = 31.83$, $P < 0.0005$) and no overall session by lesion interaction ($F(7, 126) = 1.23$, $P = 0.297$) was seen, demonstrating that both groups of rats learn over subsequent sessions at the same rate.

In order to investigate the types of errors made by the MS/VDB-lesioned rats, we looked at the errors made on either the old or the new environments during each day of the discrimination. (Because of a data recording error, we could only analyze the first 6 days of discrimination performance.) A three-way ANOVA, with session and old/new environment as within-subjects factors and lesion as a between subject factor, again revealed no significant effect of lesion ($F(1,18) = 0.011$, $P = 0.92$) and, more importantly, no old/new by lesion interaction ($F(1,18) = 0.033$, $P = 0.859$) or old/new by lesion by session interaction ($F(5,90) = 1.21$, $P = 0.313$). Main effects of session ($F(5,90) = 29.85$, $P < 0.0005$) and old/new ($F(1,18) = 15.03$, $P = 0.001$), and an interaction of old/new by session ($F(5,90) = 6.312$, $P < 0.0005$), were significant as expected. Hence, when the conditional task was introduced, control and lesioned rats performed comparably in the old environment, as well as in the new environment, although both control and lesioned rats perform better on the old environment during the first day. As a result, there was no suggestion that performance of lesioned rats was differentially disrupted in the new environment, as might be expected (Fig. 4).

We also investigated whether MS/VDB-lesioned rats had a tendency, when placed in the new environment on day 1 of conditional learning, to dig in the location of baited cup for the old environment. This would suggest that they are more likely to rely on a place map developed during initial training when the conditional discrimination is introduced. A *t*-test demonstrated no significant difference between the number of times control and MS/VDB-lesioned rats dug in the old baited cup (control mean =

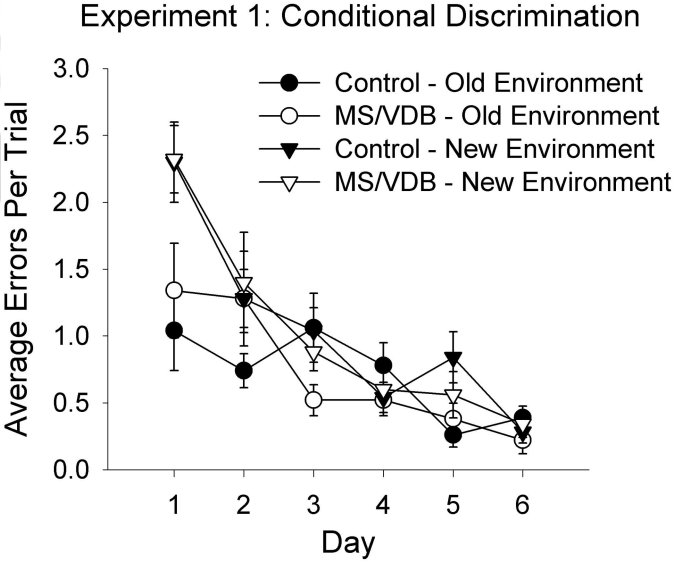

FIGURE 4. Comparison of errors in old (pretrained) and new environments in the conditional discrimination phase of Experiment 1. Control and medial septum and vertical limb of the diagonal band of Broca (MS/VDB)-lesioned rats make more errors initially in the new environment, as would be expected, and there is no differential effect of lesion in performance in either old or new environments.

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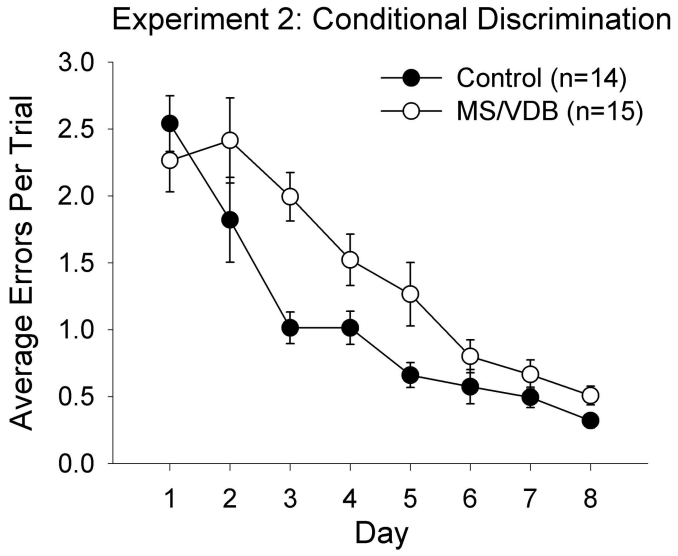

FIGURE 5. Results of Experiment 2. When the environment-spatial conditional discrimination is introduced without the rats being pretrained on one of the two environments, medial septum and vertical limb of the diagonal band of Broca (MS/VDB)-lesioned rats are impaired in simultaneously learning the location of the food reward in two different environments with different visual cues.

2.70, SEM = 0.684; MS/VDB mean = 3.80, SEM = 0.663; $t(18) = -1.55$, $P = 0.26$). Hence, it appeared that MS/VDB-lesioned rats were not disproportionately influenced by place responses developed during initial learning when a new set of environmental cues was introduced, which directed them to search in a different location.

Experiment 2: Conditional associative learning

In contrast to rats that were trained on one of the two environments before encountering the conditional discrimination, there was a significant lesion effect when rats were given concurrent trials in both environments without pretraining on a single environment (Fig. 5). MS/VDB-lesioned rats were impaired relative to control rats in performing the discrimination, and learned more slowly relative to control rats. A two-way ANOVA, with lesion as a between-subject factor and session as a within-subject factor, revealed that there was a significant effect of lesion ($F(1, 27) = 6.74$, $P = 0.015$), a significant effect of session ($F(7, 189) = 42.30$, $P < 0.0005$), and a significant session by lesion interaction ($F(7, 189) = 2.88$, $P = 0.007$).

To examine the possibility that MS/VDB-lesioned rats simply performed as well as controls on one environment and were impaired relative to controls on the other, rather then being impaired on both, we grouped the errors each rat made into the two different environments. We then determined which environment was each rat's "best" and "worst," based on the mean across all days for each environment. (Because of a data recording mechanical malfunction, the types of errors that 5 rats made on day 2 were missing, thus precluding their inclusion in this analysis and in turn making our n = 24 for this particular measure.) A three-factor ANOVA, with lesion as a between subject factor, session as a within subject factor and best/worst as a within subject factor revealed an overall lesion effect ($F(1,22) = 5.03$, $P = 0.035$), an overall effect of session ($F(7,154) = 47.43$, $P < 0.0005$) and a session by lesion interaction ($F(7,154) = 6.534$, $P < 0.0005$). Furthermore, as expected, there was an overall effect of best/worst environment ($F(1,22) = 28.175$, $P < 0.0005$) but no best/worst by lesion interaction ($F(1,22) = 0.205$, $P = 0.656$), indicating that the difference between best-performing and worst-performing environments is comparable between MS/VDB-lesioned and control rats. There was also no significant best/worst, session, and lesion interaction ($F(7,154) = 0.375$, $P = 0.916$), indicating that the difference in learning between the "best" and "worst" environments is the same for MS/VDB-lesioned and control rats (Fig. 6). In addition, both control and lesioned rats found each of the two environments, octagon or cube, equally difficult. That is there was a similar number of lesion (7) and control rats (6) that did "worse" in the cube or "worse" on the octagon environment (lesion = 7, control = 7). One should note that two control rats had an equal number of average errors per trial for both. Hence, MS/VDB-lesioned rats perform poorly relative to controls in both environments, and are not able to master one of the two components of the conditional problem.

DISCUSSION

The present study demonstrates that selective lesions of MS/VDB cholinergic neurons in rats impairs environment-spatial conditional associative learning when rats are required to acquire both

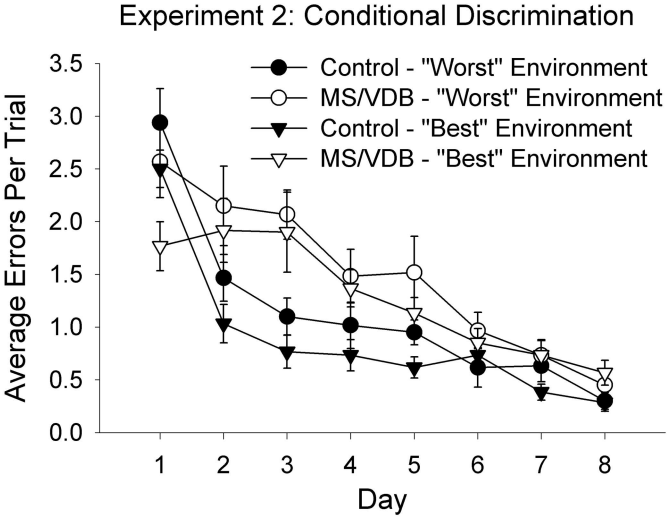

FIGURE 6. Comparison of errors in "best" and "worst" environments in the conditional discrimination in Experiment 2. When each rat is assigned a "best" and "worst" environment based on overall errors in the two environments composing the conditional discrimination, an effect of lesion on performance in both environments is still observed.

components of the conditional problem simultaneously. However, when rats are pretrained on one component of the conditional problem before being required to perform the discrimination simultaneously, MS/VDB cholinergic lesions are without effect. This suggests that although MS/VDB cholinergic neurons play an important role in conditional associative learning, rats with these lesions are capable of applying a conditional rule to solve an environment-spatial conditional problem. Our study further confirms that the function of MS/VDB cholinergic neurons is similar in rats and monkeys, because lesions of these neurons in both species produce impairments in visual-spatial conditional learning (Ridley et al. 1996, 1999; Ridley and Baker 1997).

MS/VDB-saporin lesions were without effect on learning a rewarded location in a single environment, consistent with many other studies in rats (Baxter et al., 1995; Bannon et al., 1996; Pang and Nocera, 1999; Cahill and Baxter, 2001). Navigation in this study, as in others, could not be supported by a simple egocentric strategy because rats were put into the testing apparatus facing in different directions. As mentioned previously, perhaps the reason the effects of MS/VDB cholinergic lesions have not been seen in such single environment tasks is because such tasks do not require that the rat use two place fields interchangeably. Consequently, our results from the concurrently learned discrimination (Experiment 2) seem to confirm that cholinergic MS/VDB neurons are necessary for spatial memory tasks that require the rat to switch reliably from one environmental "map" to another during learning, which requires efficient hippocampal place field re-mapping. Furthermore, this hypothesis may explain why fornix lesions sometimes spare conditional learning in rats (Sziklas et al., 1998; Sziklas and Petrides, 2002). In these tasks, the rats are performing a conditional discrimination (spatial-visual or visual-spatial) in a single environment, in which a single spatial map can be formed, because the spatial cues available to the rat are the same in both parts of the conditional problem.

The question remains of what benefit pretraining on one component of the conditional problem could have on the place fields of MS/VDB-lesioned rats, allowing these rats to perform the conditional discrimination normally. That is, it is unclear why pretraining on one of the environments would have eliminated a requirement for remapping of place fields, or switching between environmental "maps." Based on an electrophysiological recording experiment by Ikonen and colleagues (2002), which showed that rats with MS/VDB-saporin lesions have overly stable hippocampal place fields, we expected to find an impairment in these rats on concurrently learned conditional visual-spatial discriminations between two environments, regardless of whether rats had been pretrained on one environment or not. It is possible that when one environment or condition is learned first as a simple visual task, its requirement on place field representations differs.

Insight into this pattern comes from a recent study by Lever et al. (2002), which seems to explain more accurately the discrepancy between visual-spatial conditional discrimination with and without pretraining. Lever et al. (2002) recorded from the CA1 region of the rat hippocampus and showed that rats exposed to two geometrically different environments start off with similar place fields for both environments that, over time, diverge into two distinct place field maps. Once learned, divergence is even maintained after long delays. Indeed this divergence is seen as early as 5 days after concurrent exposure to both environments, which is consistent with our experimental time frame for control rats. It seems that in our study MS/VDB-lesioned rats are impaired in accomplishing this divergence when two geometrically, and visually, distinct environments must be learned and discriminated simultaneously. Neither environment has an advantage, so MS/VDB rats are not simply using one set of place fields that will predict the reward in one environment only.

This divergence-specific impairment hypothesis also suggests an explanation for why MS/VDB-lesioned rats are not significantly impaired on environment-spatial discrimination after pretraining on one environment. In studies in which a novel environment was introduced after a familiar environment was well learned, place fields initially destabilized in both control and MS/VDB-lesioned rats (Ikonen et al., 2002). Although the representation of the new environment formed by MS/VDB-lesioned rats showed similarity to that of the old environment, whereas control rats showed no such correlation, it is possible that the destabilization of place fields produced by the introduction of the new environment provides a sufficient cue to utilize the differences in the representations of the new environment to guide performance. Consistent with this view is that our MS/VDB-lesioned rats pretrained in one environment did not continue to search in the rewarded location from the pretrained environment any more than control rats when a new environment was introduced (Experiment 1). Clearly, this is a testable hypothesis via electrophysiological recording in hippocampus of rats with and without MS/VDB-cholinergic lesions that are exposed to two geometrically distinct environments in tandem. Similarly, we can probably discount the hypothesis that interference from previously established place fields is greater in MS/VDB-lesioned rats, because learning of the discrimination in one environment did not impair subsequent learning of the new discrimination when the full conditional problem was presented.

An alternative explanation of our result is that MS/VDB cholinergic lesions simply increase sensitivity to interference from learning two problems concurrently, regardless of an effect on place field properties specifically, or the extent to which place field representations govern choice behavior in the discrimination problem. It is not clear that this explanation is necessarily mutually exclusive of the place field hypothesis. Indeed, effects of MS/VDB cholinergic lesions on place field properties might provide a mechanism for MS/VDB lesion effects on this kind of interference.

Our finding suggest that alterations in place field plasticity following loss of cholinergic projections to the hippocampus may have a specific behavioral consequence, although perhaps one that is limited to an impairment in divergence between place fields rather than novel place field mapping. Furthermore, they help clarify the specific role of cholinergic medial septal neurons in spatial memory in rats. Because noncholinergic septal inputs to the hippocampus appear sufficient to sustain spatial memory (Baxter et al., 1995; Pang and Nocera, 1999; Cahill and Baxter, 2001), it appears that the cholinergic neurons are specifically needed to al-

low the divergence of place fields and efficiently form two distinct maps from one. MS/VDB-lesioned rats seem to retain the ability to switch back and forth between the representations of two environments efficiently when a stable map is formed for one of the environments before the second is introduced. These findings may also provide a new behavioral system in which to examine the consequences of cholinergic depletion on hippocampal information processing, as well as the consequences of alterations in hippocampal place cell activity for spatial cognition.

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REFERENCES

Amaral DG, Kurz J. 1985. An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. J Comp Neurol 240:37–59.

Bannon AW, Curzon P, Gunther KL, Decker MW. 1996. Effects of intraseptal injection of 192-IgG-saporin in mature and aged Long-Evans rats. Brain Res 718:25–36.

Baxter MG, Bucci DJ, Gorman LK, Wiley RG, Gallagher M. 1995. Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats. Behav Neurosci 109:714–722.

Berger-Sweeney J, Stearns NA, Frick KM, Beard B, Baxter MG. 2000. Cholinergic basal forebrain is critical for social transmission of food preferences. Hippocampus 10:729–738.

Bland BH. 1986. The physiology and pharmacology of hippocampal formation theta rhythms. Prog Neurobiol 26:1–54.

Cahill JFX, Baxter MG. 2001. Cholinergic and noncholinergic septal neurons modulate strategy selection in spatial learning. Eur J Neurosci 14:1856–1864.

Chappell J, McMahan R, Chiba A, Gallagher M. 1998. A re-examination of the role of basal forebrain cholinergic neurons in spatial working memory. Neuropharmacology 37:481–487.

Eichenbaum H, Dudchenko P, Wood E, Shapiro M, Tanila H. 1999. The hippocampus, memory, and place cells: is it spatial memory or a memory space? Neuron 23:209–226.

Freund TF. 1989. GABAergic septohippocampal neurons contain parvalbumin. Brain Res 478:375–381.

Heckers S, Ohtake T, Wiley RG, Lappi DA, Geula C, Mesulam M-M. 1994. Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. J Neurosci 14:1271–1289.

Ikonen S, McMahan R, Gallagher M, Eichenbaum H, Tanila H. 2002. Cholinergic system regulation of spatial representation by the hippocampus. Hippocampus 12:386–397.

Janis LS, Glasier MM, Fulop Z, Stein DG. 1998. Intraseptal injections of 192 IgG-saporin produce deficits for strategy selection in spatial-memory tasks. Behav Brain Res 90:23–34.

Leanza G, Muir J, Nilsson OG, Wiley RG, Dunnett SB, Bjorklund A. 1996. Selective immunolesioning of the basal forebrain cholinergic system disrupts short-term memory in rats. Eur J Neurosci 8:1535–1544.

Leutgeb S, Mizumori SJ. 1999. Excitotoxic septal lesions result in spatial memory deficits and altered flexibility of hippocampal single-unit representations. J Neurosci 19:6661–6672.

Lever C, Wills T, Cacucci F, Burgess N, O'Keefe J. 2002. Long-term plasticity in hippocampal place-cell representation of environmental geometry. Nature 416:90–94.

Lewis PR, Shute CCD, Silver A. 1967. Confirmation from choline acetylase analyses of a massive cholinergic innervation to the rat hippocampus. J Physiol 191:215–224.

McMahan RW, Sobel TJ, Baxter MG. 1997. Selective immunolesions of hippocampal cholinergic input fail to impair spatial working memory. Hippocampus 7:130–136.

Mizumori SJY, Perez GM, Alvarado MC, Barnes CA, McNaughton BL. 1990. Reversible inactivation of the medial septum differentially affects two forms of learning in rats. Brain Res 528:12–20.

Olton DS, Walker JA, Gage FH. 1978. Hippocampal connections and spatial discrimination. Brain Res 139:295–308.

Pang KCH, Nocera R. 1999. Interactions between 192-IgG saporin and intraseptal cholinergic and GABAergic drugs: role of cholinergic medial septal neurons in spatial working memory. Behav Neurosci 113: 265–275.

Rashidy-Pour A, Motamedi F, Motahed-Larijani Z. 1996. Effects of reversible inactivations of the medial septal area on reference and working memory versions of the Morris water maze. Brain Res 709:131–140.

Ridley RM, Baker HF. 1997. Evidence for a specific information processing deficit in monkeys with lesions of the septo-hippocampal system. Cortex 33:167–176.

Ridley RM, Aitken DM, Baker HF. 1989. Learning about rules but not about reward is impaired following lesions of the cholinergic projection to the hippocampus. Brain Res 502:306–318.

Ridley RM, Thornley HD, Baker HF, Fine A. 1991. Cholinergic neural transplants into hippocampus restore learning ability in monkeys with fornix transections. Exp Brain Res 83:533–538.

Ridley RM, Gribble S, Clark B, Baker HF, Fine A. 1992. Restoration of learning ability in fornix-transected monkeys after fetal basal forebrain but not fetal hippocampal tissue transplantation. Neuroscience 48: 779–792.

Ridley RM, Baker HF, Harder JA, Pearson C. 1996. Effects of lesions of different parts of the septo-hippocampal system in primates on learning and retention of information acquired before or after surgery. Brain Res Bull 40:21–32.

Ridley RM, Barefoot HC, Maclean CJ, Pugh P, Baker HF. 1999. Different effects on learning ability after injection of the cholinergic immunotoxin ME20.4IgG-saporin into the diagonal band of Broca, basal nucleus of Meynert, or both in monkeys. Behav Neurosci 113:303–315.

Rye DB, Wainer BH, Mesulam M-M, Mufson EJ, Saper CB. 1984. Cortical projections arising from the basal forebrain: a study of cholinergic and noncholinergic components employing combined retrograde tracing and immunohistochemical localization of choline acetyltransferase. Neuroscience 13:627–643.

Shapiro ML, Eichenbaum H. 1999. Hippocampus as a memory map: synaptic plasticity and memory encoding by hippocampal neurons. Hippocampus 9:365–384.

Shapiro ML, Simon DK, Olton DS, Gage FH, Nilsson O, Björklund A. 1989. Intrahippocampal grafts of fetal basal forebrain tissue alter place fields in the hippocampus of rats with fimbria-fornix lesions. Neuroscience 32:1–18.

Shen J, Barnes CA, Wenk GL, McNaughton BL. 1996. Differential effects of selective immunotoxic lesions of medial septal cholinergic cells on spatial working and reference memory. Behav Neurosci 110:1181–1186.

Sziklas V, Petrides M. 2002. Effects of lesions to the hippocampus or the fornix on allocentric conditional associative learning in rats. Hippocampus 12:543–550.

Sziklas V, Petrides M, Leri F. 1996. The effects of lesions to the mammillary region and the hippocampus on conditional associative learning by rats. Eur J Neurosci 8:106–115.

Sziklas V, Lebel S, Petrides M. 1998. Conditional associative learning and the hippocampal system. Hippocampus 8:131–137.

Vanderwolf CH. 1969. Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr Clin Neurophysiol 26:407–418.

Walsh TJ, Herzog CD, Gandhi C, Stackman RW, Wiley RG. 1996. Injection of 192 IgG-saporin into the medial septum produces cholinergic hypofunction and dose-dependent working memory deficits. Brain Res 726:69–79.

Walsh TJ, Gandhi C, Stackman RW. 1998. Reversible inactivation of the medial septum or nucleus basalis impairs working memory in rats: a dissociation of memory and performance. Behav Neurosci 112:1114–1124.

Wiley RG, Oeltmann TN, Lappi DA. 1991. Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. Brain Res 562:149–153.

Wrenn CC, Lappi DA, Wiley RG. 1999. Threshold relationship between lesion extent of the cholinergic basal forebrain in the rat and working memory impairment in the radial maze. Brain Res 847:284–298.